RealArt™ M. tuberculosis TM PCR Kit

Quantitative

***in vitro* Diagnostics**

for use with the *ABI PRISM®*

Sequence Detection Systems

(Applied Biosystems)

User Manual

February 2004

CE

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RealArt™ M. tuberculosis TM PCR Kit

for use with the *ABI PRISM® 7000*, *7700* and *7900HT Sequence Detection Systems* (Applied Biosystems)♦.

Attention: The *RealArt™ M. tuberculosis TM PCR Kit* may neither be used in combination with the *GeneAmp® 5700 SDS* nor with the 384 plate format of the *ABI PRISM® 7900HT SDS*.

1. Contents

	Labelling and contents	Art. No. 5511-01 24 reactions	Art. No. 5511-02 48 reactions	Art. No. 5511-03 96 reactions
Blue	*M. tuberculosis TM Master*	2 x 12 rxns	4 x 12 rxns	8 x 12 rxns
Yellow	*M. tuberculosis TM Mg-Sol*	1 x 400 µl	1 x 400 µl	1 x 400 µl
Red	*M. tuberculosis TM QS 1¤ 3 x 10^4 cop/µl*	1 x 200 µl	1 x 200 µl	1 x 200 µl
Red	*M. tuberculosis TM QS 2¤ 3 x 10^3 cop/µl*	1 x 200 µl	1 x 200 µl	1 x 200 µl
Red	*M. tuberculosis TM QS 3¤ 3 x 10^2 cop/µl*	1 x 200 µl	1 x 200 µl	1 x 200 µl
Red	*M. tuberculosis TM QS 4¤ 3 x 10^1 cop/µl*	1 x 200 µl	1 x 200 µl	1 x 200 µl
Green	*M. tuberculosis TM IC¤*	1 x 1000 µl	1 x 1000 µl	1 x 1000 µl
White	*Water (PCR grade)*	1 x 1000 µl	1 x 1000 µl	1 x 1000 µl

¤ QS = Quantitation Standard
IC = Internal Control

♦Applied Biosystems is a registered trademark and *ABI PRISM®* is a trademark of Applera Corporation or its subsidiaries in the US and/or certain other countries.

2. Storage

The components of the *RealArt™ M. tuberculosis TM PCR Kit* should be stored at -20°C and are stable until the expiry date stated on the label. Repeated thawing and freezing (> 2 x) should be avoided, as this may reduce the sensitivity. If the reagents are to be used only intermittently, they should be frozen in aliquots. Storage at +4°C should not exceed a period of five hours.

3. Additionally Required Materials and Devices

- Disposable powder-free gloves
- DNA isolation kit (see **8.1 DNA Isolation**)
- Lysozyme mix (see **8.1 DNA Isolation**)
- Pipettes (adjustable)
- Sterile pipette tips with aerosol barrier
- Vortex mixer
- Desktop centrifuge with rotor for 2 ml reaction tubes
- Centrifuge with rotor for microtitre plates (optional)
- 96-well reaction plate/reaction tubes for optical measurement with corresponding optical closing materials♦ (see **8.4 Preparing the PCR**)
- 96-well two-part retaining rack for use with optical reaction tubes (*96-Well Tray/Retainer Set*, Cat. No. 403 081, Applied Biosystems), see **8.4 Preparing the PCR**
- Compression pad for use with optical adhesive covers (*Optical Cover Compression Pads*, Cat. No. 4 312 639, Applied Biosystems), see **8.4 Preparing the PCR**
- Applicator for sealing of the reaction plates using optical adhesive covers (*Adhesive Seal Applicator Kit*, Cat. No. 4 333 183, Applied Biosystems)

♦The use of reaction tubes for optical analyses with domed caps is only permitted with the *ABI PRISM® 7700 SDS* and requires an adjustment of the exposure time (see **8.5.2 Programming the *ABI PRISM® 7700 SDS***, 8.5.2.5 Important Additional Settings).

- *ABI PRISM® 7000*, *7700* or *7900HT SDS* (Applied Biosystems)

Attention: A valid calibration of the pure dyes (*Pure Spectra Component File*) and the background (*Background Component File*) is necessary when putting the instruments into operation.

4. General Precautions

The user should always pay attention to the following:

- Use pipette tips with filters.
- Extract and store positive material (specimens, controls and amplicons) separately from all other reagents and add it to the reaction mix in a spatially separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the cooling block.

5. Pathogen Information

Mycobacteria are distributed worldwide. It is estimated that one third of the world's population is infected with *Mycobacterium tuberculosis*, the pathogen of tuberculosis. About eight million new infections and about three million deaths are reported each year. Countries in the third world are largely affected, but due to resistance development and the importation of infections, tuberculosis incidents occur increasingly often in industrialized nations. The homeless, drug addicted and immunocompromised persons are particularly at risk of infection. Transmission of the bacteria occurs via aerosols, with an increased risk of infection in cases of active tuberculosis. Tuberculosis is a chronic, cyclic disease. In the primary stage, mainly parts of the lungs and the associated lymph nodes are affected. Depending on the immune status of the patient, however, mycobacteria can colonize in the entire lung and other organs. In cases of immunosuppression the infection may spread from these sites and, even years later, can be reactivated (recrudescent). Signs of such a

reactivation in the lungs include chronic fever, weight loss, night sweats and coughing. Other symptoms depend on the affected organs.

6. Principle of Real-Time PCR

Pathogen diagnosis by the polymerase chain reaction (PCR) is based on the amplification of specific regions of the pathogen genome. In real-time PCR, the amplified product is detected via fluorescent dyes. These are usually linked to oligonucleotide probes which bind specifically to the amplified product. Monitoring the fluorescence intensities during the PCR run (i. e. in real-time) allows the detection and quantitation of the accumulating product without having to re-open the reaction tubes after the PCR run.

7. Product Description

The *RealArt™ M. tuberculosis TM PCR Kit* constitutes a ready-to-use system for the detection of DNA of all members of the *M. tuberculosis* complex (*M. tuberculosis*, *M africanum I + II*, *M bovis*, *M bovis BCG*, *M. microti*, *M. canettii*) using polymerase chain reaction (PCR) in the *ABI PRISM® 7000*, *7700* and *7900HT Sequence Detection System* (Applied Biosystems). The *M. tuberculosis TM Master* contains reagents and enzymes for the specific amplification of a 163 bp region of the mycobacterial genome. The amplicon is detected by measuring the FAM fluorescence in the *ABI PRISM® SDS*. In addition, the *RealArt™ M. tuberculosis TM PCR Kit* contains a second heterologous amplification system to identify possible PCR inhibition. This is detected as an *Internal Control* (IC) by measuring the JOE fluorescence. The detection limit of the analytical *M. tuberculosis* complex PCR (see **11.1 Sensitivity**) is not reduced. External positive controls (*M. tuberculosis TM QS 1 - 4*) are supplied which allow the determination of the pathogen load. For further information, please refer to section **8.3 Quantitation.**

8. Protocol

8.1 DNA Isolation

Before the DNA isolation, large sample volumes or strongly acidic samples must first be concentrated or neutralized, respectively. For the analysis of sputum, we recommend a NALC-NaOH decontamination; stomach fluids should be neutralized with phosphate buffer. After a final centrifugation, the bacteria pellet can be used for the following DNA isolation (for more details contact our technical support at techsupport@artus-biotech.com).

Various manufacturers offer DNA isolation kits. Sample volumes for the DNA isolation procedure depend on the protocol used. Please carry out the DNA isolation according to the manufacturer's instructions. The following isolation kits are recommended:

Sample Material	Nucleic Acid Isolation Kit	Catalogue Number	Manufacturer	Carrier DNA/RNA
Sputum, BAL, bronchial secretion, CSF, stomach fluid, peritoneal punction	*PureArt™ DNA Mini Kit* (50)♦	90204-50	artus	not included
	QIAamp DNA Mini Kit (50)	51 304	QIAGEN	not included

Attention: It is important to follow the instructions in **Protocol D** (Protocols for bacteria) described in the *QIAGEN Kit User Manual*. We recommend a digestion with lysozyme (lysozyme mix: 20 mg/ml lysozyme, 20 mM Tris-HCl, 2 mM EDTA, 1.2 % Triton®) for at least one hour. The final DNA elution should be performed once using 100 µl AE buffer.

- If the selected isolation kit does not contain **carrier DNA/RNA**, please note that the addition of carrier RNA (RNA-Homopolymer Poly(A), Amersham Biosciences) at a concentration of 10 µg/ml lysis buffer to the sample/lysis buffer mixture is strongly recommended for extraction of nucleic acids from cell free body fluids and material low in DNA/RNA content.

♦Produced by QIAGEN GmbH, D-40724 Hilden for artus GmbH. Legal manufacturer: artus GmbH, D-22767 Hamburg.

- When using isolation protocols with **ethanol**-containing washing buffers, please carry out an additional centrifugation step before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.
- The *RealArt™ M. tuberculosis TM PCR Kit* should not be used with **phenol**-based isolation methods.

Important: The *Internal Control* of the *RealArt™ M. tuberculosis TM PCR Kit* can be used directly in the isolation procedure (see **8.2 Internal Control**).

8.2 Internal Control

An *Internal Control* (*M. tuberculosis TM IC*) is supplied. This allows the user **both to control the isolation procedure and to check for possible PCR inhibition**. For this application, add the *Internal Control* to the isolation at a ratio of 0.1 µl per 1 µl elution volume. For example, using the QIAamp DNA Mini Kit (QIAGEN) the DNA is eluted in 100 µl AE buffer. Hence, 10 µl of the *Internal Control* should be added initially. The quantity of IC used depends **only** on the elution volume. Please note that the IC should be added to the mixture of lysis buffer and sample material. Please do **not** add the IC to the lysis buffer or sample material directly!

The IC can optionally be used **exclusively to check for possible PCR inhibition**. For this application, add 0.5 µl of the IC and 2 µl *M. tuberculosis TM Mg-Sol* per test mixture directly to 13 µl *M. tuberculosis TM Master*. For each PCR reaction use 15 µl of the Master Mix produced as described above[♦], then add 10 µl of the purified sample. If you are preparing a PCR run for several samples please increase the volume of the *M. tuberculosis TM Master*, the *M. tuberculosis TM Mg-Sol* and the *Internal Control* according to the number of samples (see **8.4 Preparing the PCR**).

[♦]The volume increase caused by adding the IC is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

8.3 Quantitation

The enclosed quantitation standards (*M. tuberculosis TM QS 1 - 4*) are treated as previously purified samples and the same volume is used (10 µl). To generate a standard curve in an *ABI PRISM® Sequence Detection System*, all four quantitation standards should be used and defined as standards with specification of the corresponding concentrations (see **8.5 Programming the *ABI PRISM® SDS***). The import of standard curves from previous runs is not possible with the *ABI PRISM® 7000*, *7700* and *7900HT SDS* software.

Attention: The quantitation standards are defined as copies/µl. The following equation has to be applied to convert the values determined using the standard curve into copies/ml of sample material:

$$\text{Result (copies / ml)} = \frac{\text{Result (copies / µl)} \times \text{Elution Volume (µl)}}{\text{Sample Volume (ml)}}$$

If a concentration of the sample material was performed by e. g. centrifugation prior to nucleic acid extraction, please enter the entire initial sample volume in the above equation.

8.4 Preparing the PCR

Prepare the number of required reaction tubes or a 96-well reaction plate for the scheduled reactions. Recommended materials are listed in the following table:

Article	Description	Catalogue Number	Manufacturer	Retaining Rack♦	Compression Pad
96-well optical reaction plate	96-Well Optical Reaction Plate	4 306 737	Applied Biosystems	no	-
Optical adhesive cover	Optical Adhesive Covers	4 311 971	Applied Biosystems	-	yes
Optical reaction tubes	*ABI PRISM*® Optical Tubes, 8 Tubes/Strip	4 316 567	Applied Biosystems	yes	-
Optical reaction tubes	MicroAmp® Optical Tubes	N8010933	Applied Biosystems	yes	-
Optical cap (flat)	*ABI PRISM*® Optical Caps, 8 Caps/Strip	4 323 032	Applied Biosystems	-	no

Attention: The use of reaction tubes for optical analyses with domed caps is only permitted with the *ABI PRISM® 7700 SDS* and requires an adjustment of the exposure time (see **8.5.2 Programming the *ABI PRISM® 7700 SDS***, 8.5.2.5 Important Additional Settings).

When preparing the PCR reaction, please make sure that at least one quantitation standard per PCR run as well as one negative control (*Water, PCR grade*) are performed in parallel. To generate a standard curve, use all supplied quantitation standards (*M. tuberculosis TM QS 1 - 4*) for each PCR run. Before each use, all reagents need to be thawed completely, mixed (by repeated up and down pipetting or by quick vortexing) and centrifuged briefly.

♦If the two-part retaining rack is used, it is necessary to open the reaction tubes when inserting them into and removing them from the rack. In order to avoid contamination due to this procedure, please use the lower part of the retaining rack only.

If you want to use the *Internal Control* **to monitor the isolation procedure and to check for possible PCR inhibition,** the IC has already been added to the isolation (see **8.2 Internal Control**). In this case, please use the following pipetting scheme (for a schematic overview see Fig. 1):

	Number of samples	**1**	**12**
1. Preparation of Master Mix	*M. tuberculosis TM Master*	13 µl	156 µl
	M. tuberculosis TM Mg-Sol	2 µl	24 µl
	M. tuberculosis TM IC	0 µl	0 µl
	Total volume	**15 µl**	**180 µl**
2. Preparation of PCR assay	Master Mix	15 µl	15 µl each
	Sample	10 µl	10 µl each
	Total volume	**25 µl**	**25 µl each**

If you want to use the IC **exclusively to check for PCR inhibition**, the IC must then be added directly to the *M. tuberculosis TM Master*. In this case, please use the following pipetting scheme (for a schematic overview see Fig. 2):

	Number of samples	**1**	**12**
1. Preparation of Master Mix	*M. tuberculosis TM Master*	13 µl	156 µl
	M. tuberculosis TM Mg-Sol	2 µl	24 µl
	M. tuberculosis TM IC	0.5 µl	6 µl
	Total volume	**15.5 µl**♦	**186 µl**♦
2. Preparation of PCR assay	Master Mix	15 µl♦	15 µl each♦
	Sample	10 µl	10 µl each
	Total volume	**25 µl**	**25 µl each**

Pipet 15 µl of the Master Mix into each required reaction tube or well of the 96-well reaction plate. Subsequently add 10 µl of the eluate from the DNA isolation. Mix the solution well by repeated up and down pipetting. Close the reaction tubes with the corresponding caps or, alternatively, when using a 96-well reaction plate, with an optical adhesive cover (*optical adhesive covers*, Applied Biosystems). Centrifuge the reaction tubes (in a storage rack for PCR

♦The volume increase caused by adding the IC is neglected when preparing the PCR assay. The sensitivity of the detection system is not impaired.

tubes) or the 96-well reaction plate in a centrifuge with a microtitre plate rotor for 30 seconds at 1780 x g (4000 rpm) in order to collect the prepared reaction volume in the bottom of the tubes or wells. If such a centrifuge is not at your disposal, please make sure that both the Master Mix and the sample volume are pipetted to the bottom of the tubes or wells. Store the prepared reactions at +4°C until the *ABI PRISM® SDS* Instrument is programmed (see **8.5 Programming the *ABI PRISM® SDS***) and subsequently transfer them into the instrument.

Attention:

- When using optical reaction tubes in combination with optical caps, always insert a retaining rack (*96-Well Tray/Retainer Set*, Applied Biosystems) into the instrument (*ABI PRISM® 7000*, *7700* and *7900HT SDS*). If the two-part retaining rack is used, it is necessary to open the reaction tubes when inserting them into and removing them from the rack. In order to avoid contamination due to this procedure, please use the lower part of the retaining rack only.
- Using 96-well optical reaction plates in combination with optical adhesive covers requires covering by a compression pad (*Optical Cover Compression Pads*, Applied Biosystems).

Addition of the *Internal Control* to the Purification Procedure

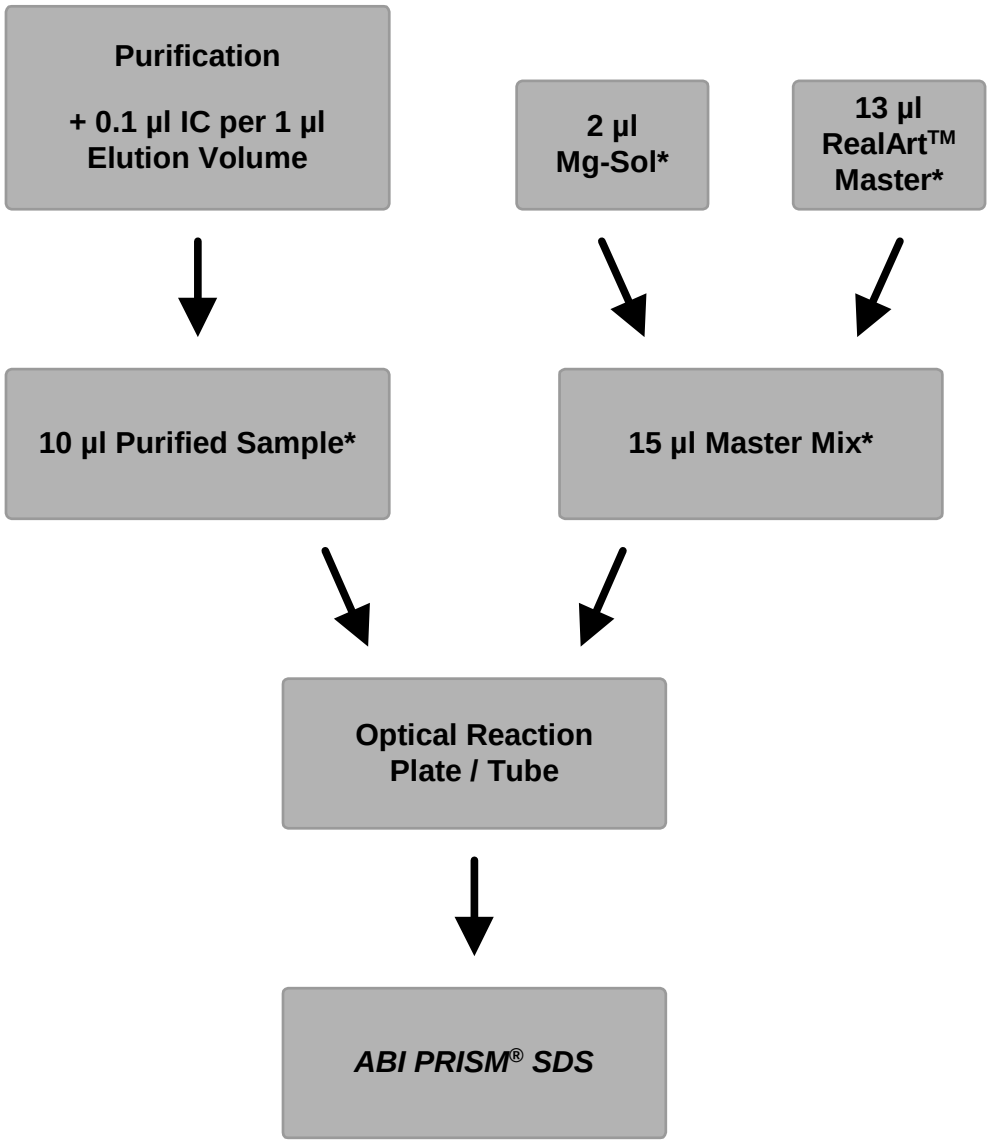
Purification
+ 0.1 µl IC per 1 µl Elution Volume
2 µl Mg-Sol*
13 µl RealArt™ Master*
10 µl Purified Sample*
15 µl Master Mix*
Optical Reaction Plate / Tube
ABI PRISM® SDS

Fig. 1: Schematic workflow for the control of both the purification procedure and PCR inhibition.

* Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

Addition of the *Internal Control* into the RealArt™ Master

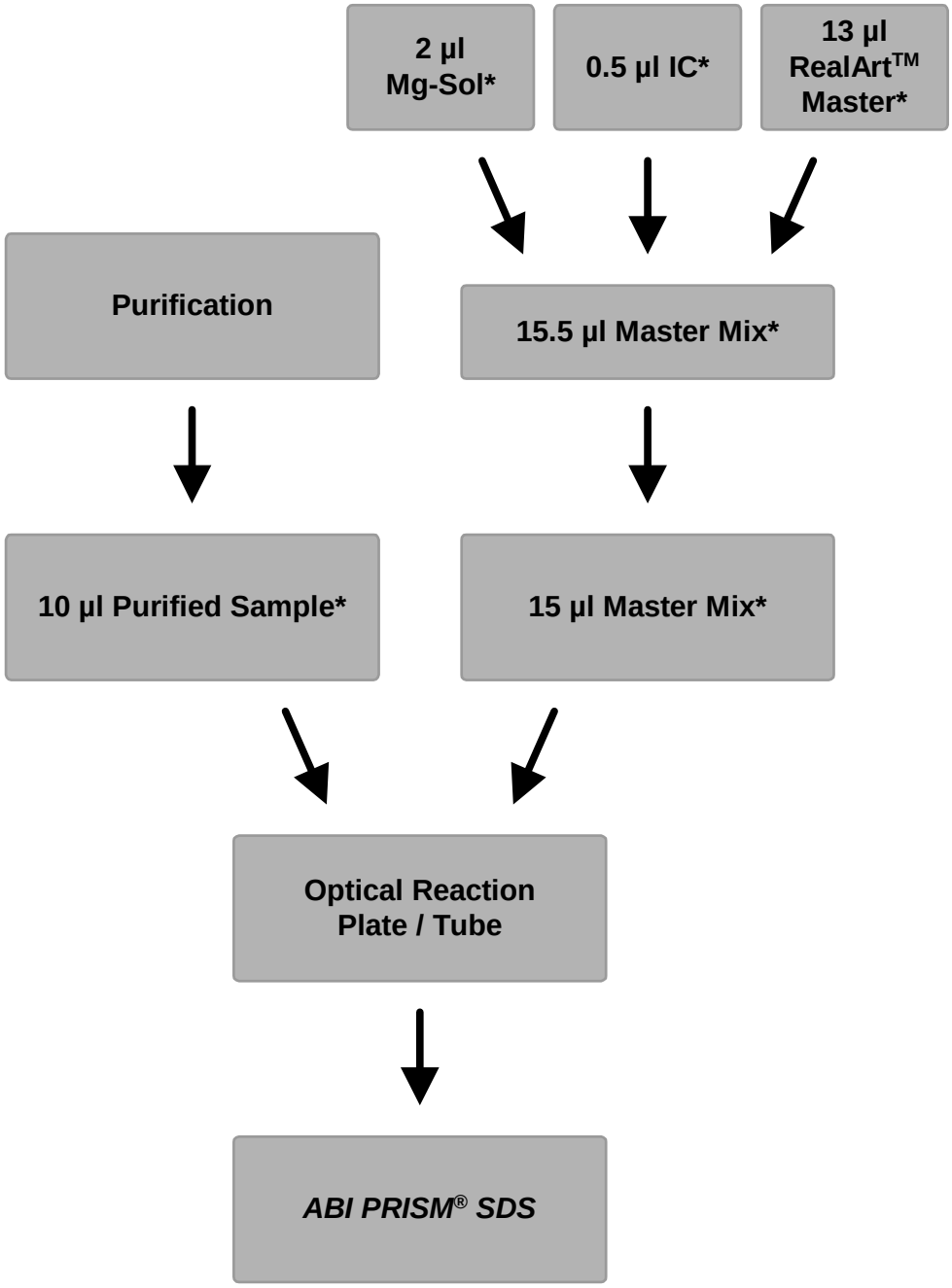

Fig. 2: Schematic workflow for the control of PCR inhibition.

* Please make sure that the solutions are thawed completely, mixed well and centrifuged briefly.

8.5 Programming the *ABI PRISM® SDS*

The software of the *ABI PRISM® 7000*, *7700* and *7900HT Sequence Detection Systems* (*SDS*) requires some additional information before starting the PCR run. The programming procedures of the instruments, however, differ considerably from each other, which is why they are treated in separate chapters as follows.

8.5.1 Programming the *ABI PRISM® 7000 SDS*

For the detection of the *M. tuberculosis* complex DNA, create a profile on your *ABI PRISM® 7000 SDS* according to the following six steps (8.5.1.1 - 8.5.1.6). All specifications refer to the *ABI PRISM® 7000 SDS Software Version 1.0.1*. For programming details of the *ABI PRISM® 7000 SDS* please refer to the *ABI PRISM® 7000 SDS User Guide*. For a better overview, the software settings are framed in bold black.

8.5.1.1 Pre-Settings for Creating a New File

Select the item *New* from the *File* menu on the *ABI PRISM® 7000 SDS* and program the following initial settings for the new file (Fig. 3). A backup template (*SDS Template* [**.sdt*]) is available from the *Template* list or by selection using the *Browse* function (see **8.5.1.5 Saving the PCR Run**). Confirm your settings (*OK*).

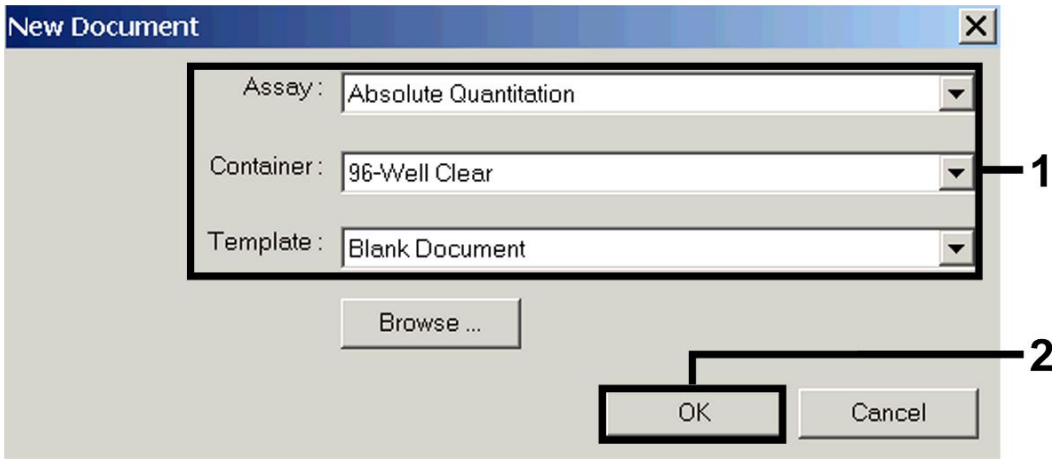

Fig. 3: Pre-settings for creating a new file (*New Document*).

8.5.1.2 Creating/Selecting the Detectors

With the help of the submenu *Detector Manager* from the *Tools* menu, assign the corresponding dyes to the file. For the detection of *M. tuberculosis* complex DNA as well as the *Internal Control* by means of the *RealArt™ M. tuberculosis TM PCR Kit*, the reporters/quenchers listed in the following table are to be defined:

Detection	Reporter	Quencher
M. tuberculosis complex DNA	FAM	none
Internal Control (*M. tuberculosis TM IC*)	JOE	TAMRA

To create these detectors, select the option *File* (bottom left of the *Detector Manager*) and subsequently the option *New*.

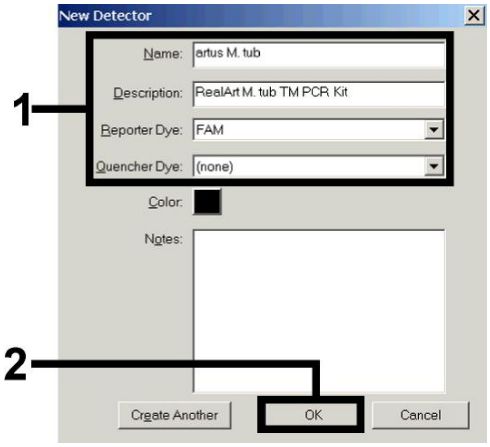

Fig. 4: Creating the *M. tuberculosis* complex specific detector (*Detector Manager*).

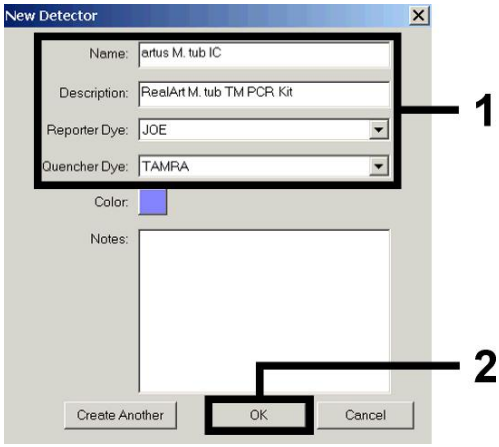

Fig. 5: Creating the IC specific detector (*Detector Manager*).

For the detection of *M. tuberculosis* complex DNA define the reporter/quencher combination **FAM/none** in the now appearing window. For the detection of the *Internal Control*, select the combination **JOE/TAMRA** (as shown in Fig. 4 and Fig. 5). By confirming the input data (*OK*), return to the *Detector Manager*. Mark the newly created detectors and transfer each selection to the *Well Inspector* by clicking on the option *Add to Plate Document* (see Fig. 6). Close the window (*Done*).

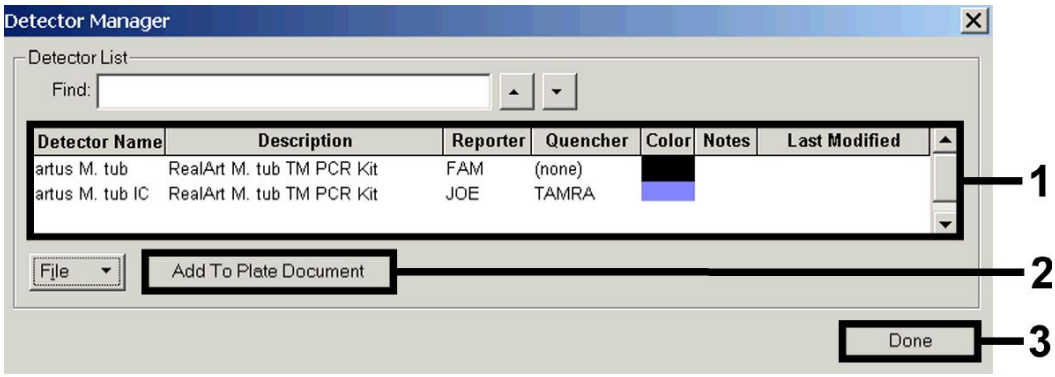

Fig. 6: Selecting the detectors (*Detector Manager*).

8.5.1.3 Assigning the Necessary Information to the Plate Positions

Open *Well Inspector* from the *View* menu to find the detectors selected under 8.5.1.2 (see Fig. 7).

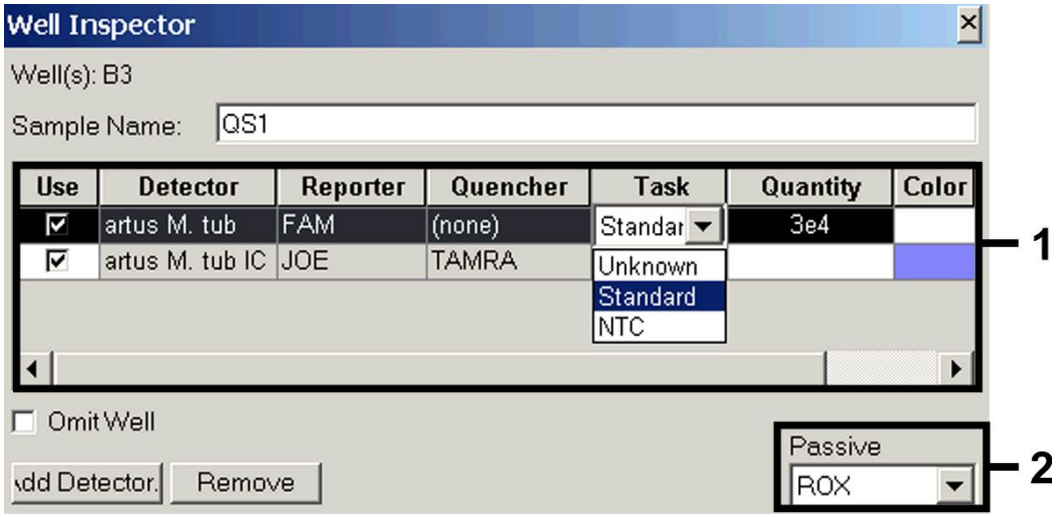

Fig. 7: Assigning the necessary information to the plate positions (*Well Inspector*).

Mark the plate positions reserved for the detection of *M. tuberculosis* complex DNA. Assign the selected detectors to these positions by activating the *Use* option of both detectors, upon which a tick will appear. For the denomination of each single reaction, select the corresponding position on the plate and enter the name (*Sample Name*). Please note that reactions with an identical *Sample Name* and an identical detector assignment will be identified as replicates by the software and will be averaged with respect to the quantified pathogen load. Then select the corresponding function (*Task*) for each sample type according to the following table:

Sample Type	Function (*Task*)	Concentration (*Quantity*)	Reporter	Quencher
Sample	Unknown	-	FAM	none
Non-template control	NTC	-	FAM	none
Standard	Standard	see **1. Contents**	FAM	none

To generate a standard curve, use all supplied quantitation standards (*M. tuberculosis TM QS 1 - 4*) per PCR run and enter the corresponding concentrations (see **1. Contents**) for each standard (*Quantity*). Please note

that for a PCR run with the *RealArt™ M. tuberculosis TM PCR Kit*, **ROX** has to be set as a passive reference (*Passive*). The equal distribution of the ROX dye in all PCR preparations of a lot by mixing of the *M. tuberculosis TM Master* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

8.5.1.4 Creating the Temperature Profile

To create a temperature profile, switch from the *Setup* level to the *Instrument* level in the software. Enter the temperature profile specific for the detection of *M. tuberculosis* complex DNA according to Fig. 8. In order to remove the 50°C step stored in the pre-settings, mark it with the left mouse button simultaneously holding the shift key, and subsequently delete it by using the backspace key. Make sure that the reaction volume for the detection of *M. tuberculosis* complex DNA using the *RealArt™ M. tuberculosis TM PCR Kit* is set to 25 µl. The option *9600 Emulation* should be activated, the pre-settings of the *Auto Increment* should remain unchanged (*Auto Increment*: 0.0°C, 0.0 Seconds).

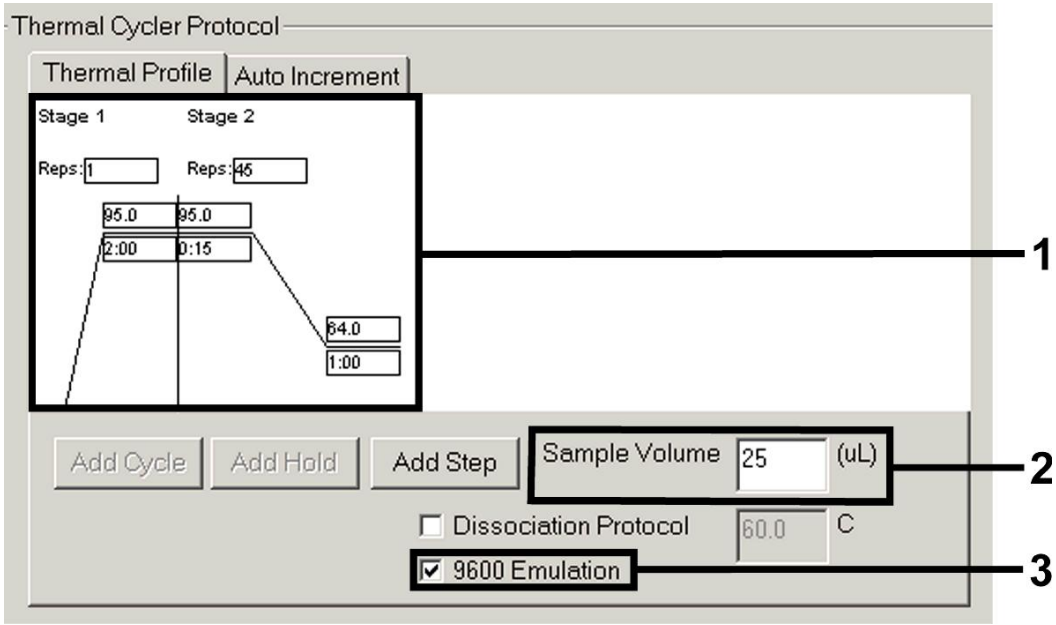

Fig. 8: Creating the temperature profile.

8.5.1.5 Saving the PCR Run

Save the settings (*Setup*) as a template in order to make use of them again later in a modified or unchanged form. By saving the *Template* file as *SDS Template* (**.sdt*) in the *Template Directory* (*Local Disk* [*C:*]*Program Files*\ *ABI PRISM 7000**Templates*, created by Applied Biosystems) this file may be selected directly in the *New Document* window from the *Template* drop-down list. Copies stored in other folders have to be opened via *Browse*. Before starting the PCR run, save it again as an *SDS Document* (**.sds*), in order to guarantee the saving of the data that will be collected during the course of the PCR.

8.5.1.6 Starting the PCR Run

Start the PCR run by selecting the option *Start* from the menu item *Instrument* or the field *Start* on the *Instrument* level.

8.5.2 Programming the *ABI PRISM® 7700 SDS*

For the detection of *M. tuberculosis* complex DNA, create a profile on your *ABI PRISM® 7700 SDS* according to the following seven steps (8.5.2.1 - 8.5.2.7). All specifications refer to the *ABI PRISM® 7700 SDS Software Version 1.9.1.* For details of programming the *ABI PRISM® 7700 SDS*, refer to the *ABI PRISM® 7700 SDS User`s Manual*. For a better overview, the software settings are framed in bold black.

8.5.2.1 Pre-settings for Creating a New File

Select the item *New Plate* from the *File* menu on the *ABI PRISM® 7700 SDS* and program the following initial settings for the new file (Fig. 9). Confirm the pre-settings (*OK*).

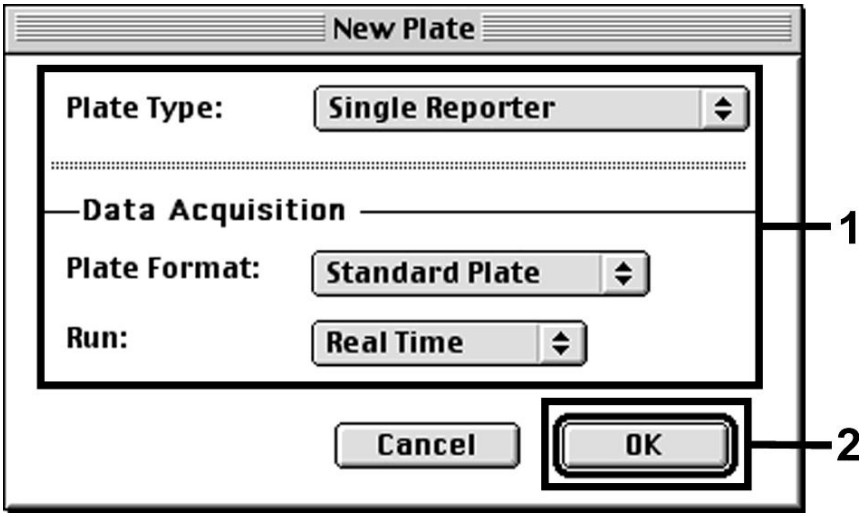

Fig. 9: Pre-settings for creating a new file (*New Plate*).

8.5.2.2 Selecting the Fluorescence Dyes and Assigning the Sample Type

With the help of the *Sample Type Setup* (*Setup* level: *Sample Type*/*Sample Type Setup*) assign the corresponding detector dyes and the corresponding sample type to the file. For the detection of *M. tuberculosis* complex DNA as well as the *Internal Control* by means of the *RealArt™ M. tuberculosis TM PCR Kit*, the reporters/quenchers listed in the following table are to be defined:

Detection	Reporter	Quencher
M. tuberculosis complex DNA	FAM	TAMRA
Internal Control (*M. tuberculosis TM IC*)	JOE	TAMRA

For the analysis of *M. tuberculosis* complex DNA by means of the *RealArt™ M. tuberculosis TM PCR Kit*, select the reporter dye **FAM** as given in the table. This applies to standards (STND) and samples (UNKN) as well as to non-template controls (UNKN). For the analysis of the *Internal Control* (IPC+), define **JOE** as reporter. As quencher set **TAMRA**. The assignment of the dyes and sample types in the window *Sample Type Setup* is shown in Fig. 10.

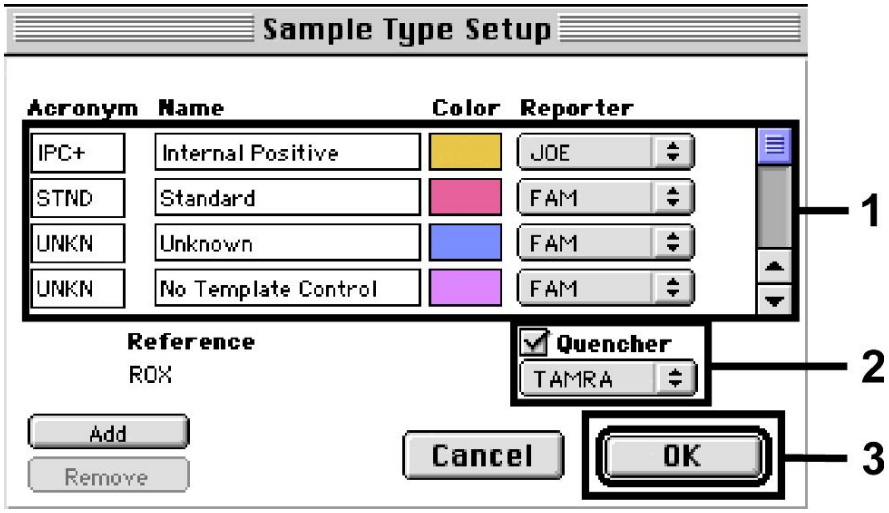

Fig. 10: Selecting the fluorescence dyes and assigning the sample type (*Sample Type Setup*).

Please assign the sample type to a corresponding function (*Acronym*) according to the following table:

Sample Type	Function (*Acronym*)	Concentration (*Quantity*)	Reporter	Quencher
Sample	UNKN	-	FAM	TAMRA
Non-template control	UNKN	-	FAM	TAMRA
Standard	STND	see **1. Contents**	FAM	TAMRA

8.5.2.3 Assigning the Necessary Information to the Plate Positions

For the assignment of the detectors and sample types to the individual plate positions, select the corresponding fields. Then open the dialogue window *Dye Layer* on the *Setup* level and assign the corresponding reporter. Upon activation of the pop-up menu *Sample Type*, you will find the sample types assigned to the reporter in the *Sample Type Setup* in the appearing list (see Fig. 11). Select the adequate sample type (see Table under 8.5.2.2) and assign the remaining plate positions by means of the *Dye Layers* and the *Sample Type* menu. A name may be assigned to each sample in the field *Sample Name*. Fields defined as *Replicates* (entering of the name of the reference sample into the *Replicate* column) are averaged by the software with respect to the quantified pathogen load and the standard deviation will be calculated.

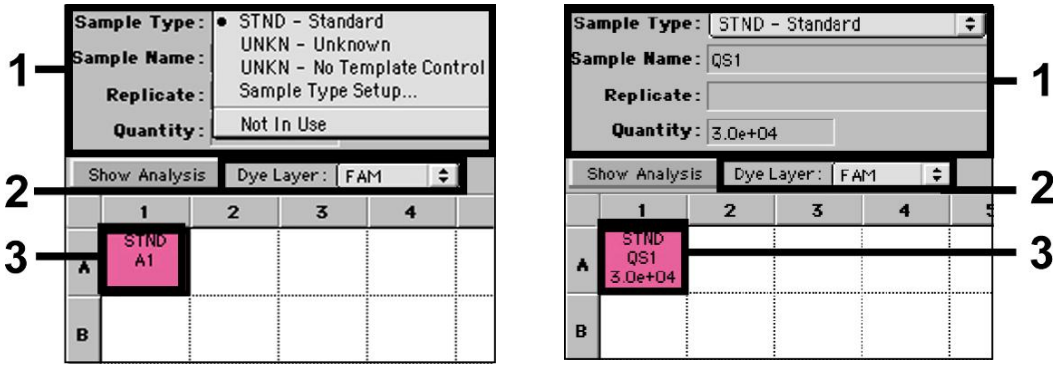

Fig. 11/12: Assigning the necessary information to the plate positions.

To generate a standard curve, use all supplied quantitation standards (*M. tuberculosis TM QS 1 - 4*) per PCR run and enter the corresponding concentrations (see **1. Contents**) for each standard into the field *Quantity* (see Fig. 12). This is only possible, however, if the positions reserved for standards were defined as such before by means of the *Sample Type* menu.

8.5.2.4 Creating the Temperature Profile

To create a temperature profile, switch to the *Thermal Cycler Conditions* menu on the *Setup* level. Enter the temperature profile special for the detection of *M. tuberculosis* complex DNA according to Fig. 13. Make sure that the reaction volume for the detection of *M. tuberculosis* complex DNA by using the *RealArt™ M. tuberculosis TM PCR Kit* is set to 25 µl. The pre-settings of the *Ramp* times and of the *Auto Increment* remain unchanged (*Ramp Time*: 0:00, *Auto Increment*: 0.0°C, 0.0 Seconds).

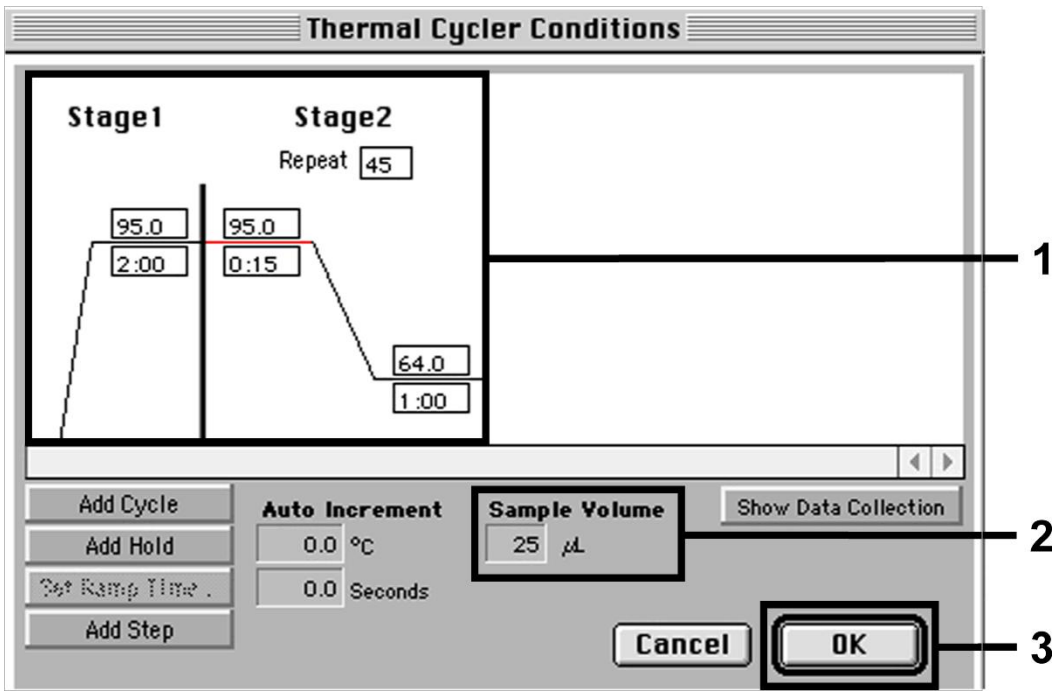

Fig. 13: Creating the temperature profile.

In addition, the *Thermal Cycler Conditions* menu contains the option *Show Data Collection*. By selecting this option the window depicted in Fig. 14 is opened. Each ramp and each plateau temperature shows a *Data Collection* icon, which illustrates the data being collected at this stage of the run. Remove all symbols except the one for the *Annealing-Extension* step (*Stage2*/Step2) in order to exclude unnecessary fluorescence measurements. The total run time and the data quantity will thus be kept at a minimum.

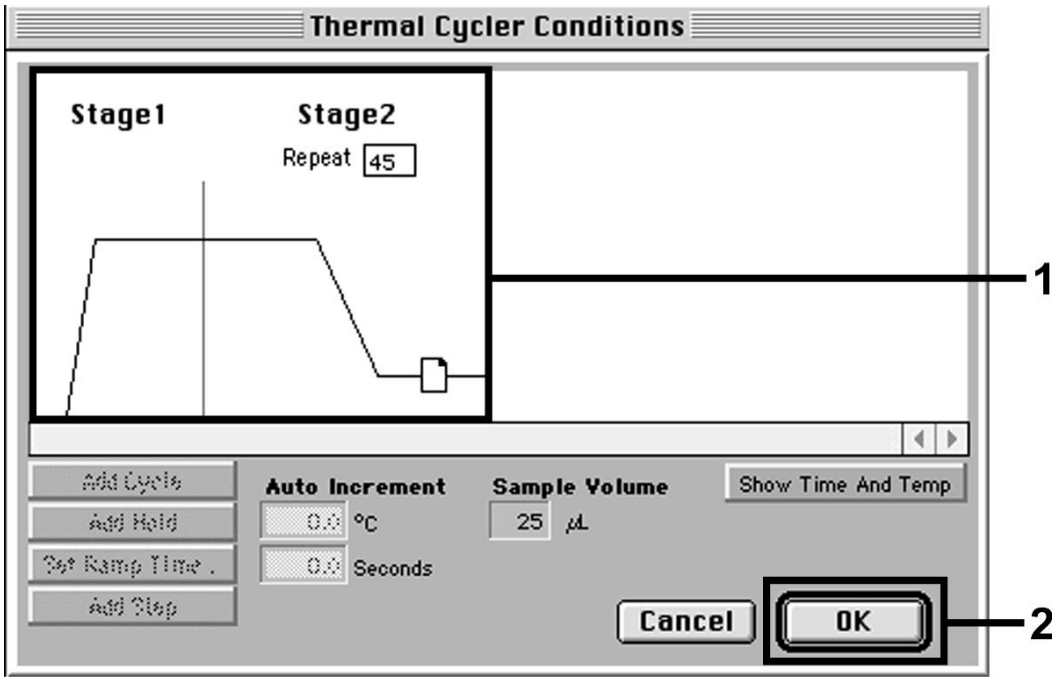

Fig. 14: Data collection.

8.5.2.5 Important Additional Settings

For the setting of the exposure time (excitation of the fluorescence dyes) as well as for the selection of the *Pure Spectra/Background* files, switch from the *Setup* level to the *Analysis* level. Select the activated sub-item *Advanced Options* to be found in the *Instrument* menu under *Diagnostics*. Adjust the settings according to Fig. 15. By inactivating the optional function *Spectra Components* (*Analysis*) the actual calibration files stored in the file *Spectra Components* at the moment of data generation are automatically used when reevaluating already analysed runs. For an analysis of previous runs by using newly entered *Spectra Components*, activate these two fields. Please note that for a PCR run with the *RealArt™ M. tuberculosis TM PCR Kit*, **ROX** has to be set as a passive reference (*Reference*). The equal distribution of the ROX dye in all PCR preparations of a lot by mixing of the *M. tuberculosis TM Master* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

Attention: When using 96-well reaction plates for optical measurements in combination with optical adhesive covers or optical reaction tubes with flat

caps, the exposure time is ten milliseconds. If you use **optical reaction tubes with domed caps**, you have to adjust this exposure time to **25 milliseconds**.

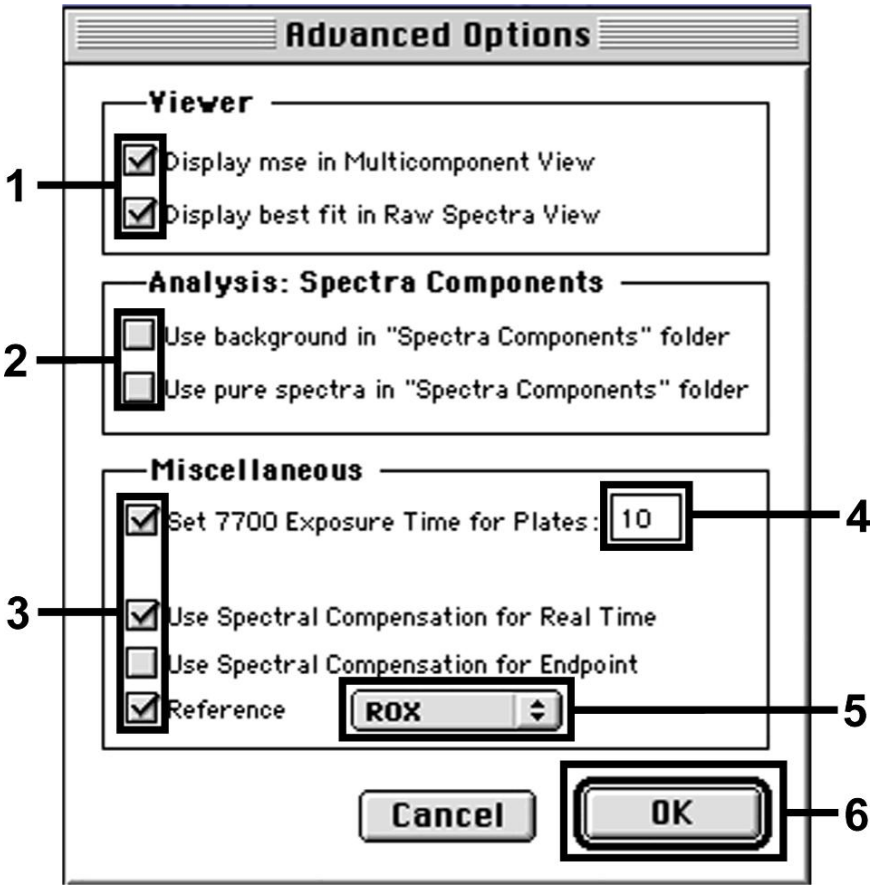

Fig. 15: Important additional settings (*Advanced Options*).

8.5.2.6 Saving the PCR Run

Save the settings (*Setup*) as a template in order to make use of them again later in a modified or unchanged form. For this purpose store the file in the *Stationary File Format*. Before starting a newly programmed PCR run, please save it again in the *Normal File Format* in order to guarantee the saving of data that will be collected during the course of the PCR.

8.5.2.7 Starting the PCR Run

Start the PCR run by selecting the option *Run* from the menu item *Instrument* or the field *Run* on the *Analysis* level.

8.5.3 Programming the *ABI PRISM® 7900HT SDS*

For the detection of *M. tuberculosis* complex DNA, create a profile on your *ABI PRISM® 7900HT SDS* according to the following six steps (8.5.3.1 - 8.5.3.6). All specifications refer to the *ABI PRISM® 7900HT SDS Software Version 2.1*. For details of programming the *ABI PRISM® 7900HT SDS*, refer to the *ABI PRISM® 7900HT SDS User Guide*. For a better overview, the software settings are framed in bold black.

8.5.3.1 Pre-Settings for Creating a New File

Select the item *New* from the *File* menu on the *ABI PRISM® 7900HT SDS* and program the following initial settings for the new file (Fig. 16). A backup template (*ABI PRISM® SDS Template Document* [**.sdt*]) is available from the *Template* list or by selection using the *Browse* function (see **8.5.3.5 Saving the PCR Run**). Confirm the pre-settings (*OK*).

Attention: The *RealArt™ M. tuberculosis TM PCR Kit* may not be used in combination with the 384 plate format of the *ABI PRISM® 7900HT SDS*.

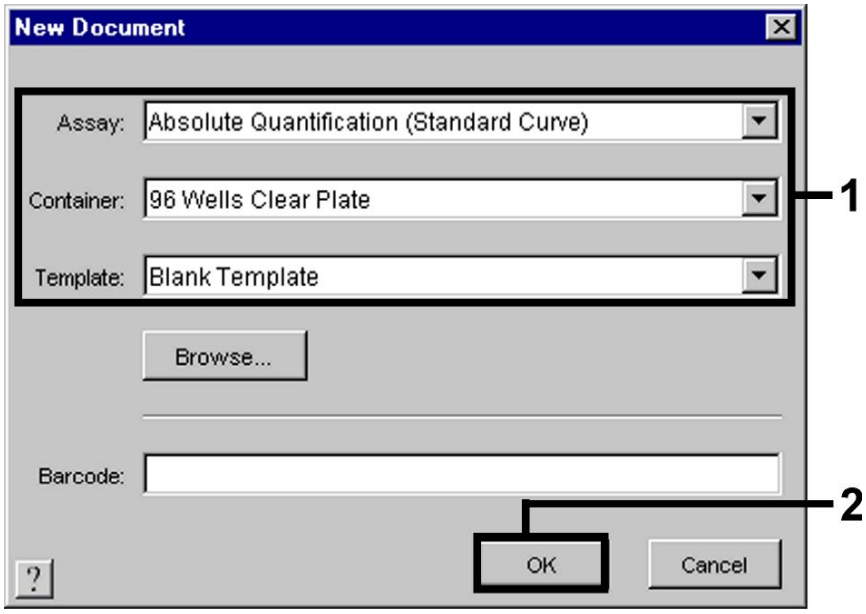

Fig. 16: Pre-settings for creating a new file (*New Document*).

8.5.3.2 Creating/Selecting the Detectors

With the help of the submenu *Detector Manager* from the *Tools* menu (alternatively: *Setup* level/*Add Detector* select function), assign the corresponding detector dyes to the file. For the detection of *M. tuberculosis* complex DNA as well as the *Internal Control* by means of the *RealArt™ M. tuberculosis TM PCR Kit*, the reporters/quenchers listed in the following table are to be defined:

Detection	Reporter	Quencher
M. tuberculosis complex DNA	FAM	Non Fluorescent
Internal Control (*M. tuberculosis TM IC*)	JOE	TAMRA

To create these detectors, select the option *New* (bottom left of the *Detector Manager*).

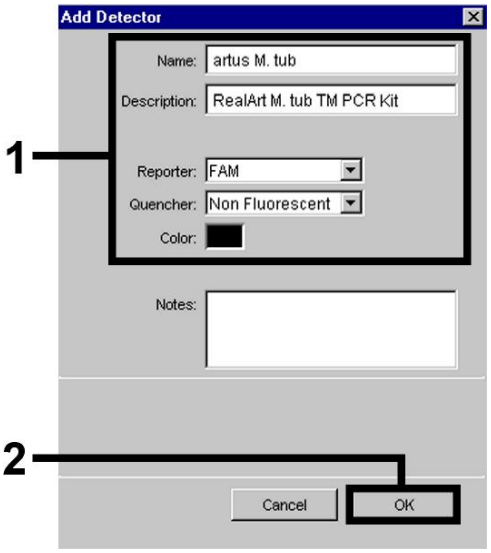

Fig. 17: Creating the *M. tuberculosis* complex specific detector (*Detector Manager*).

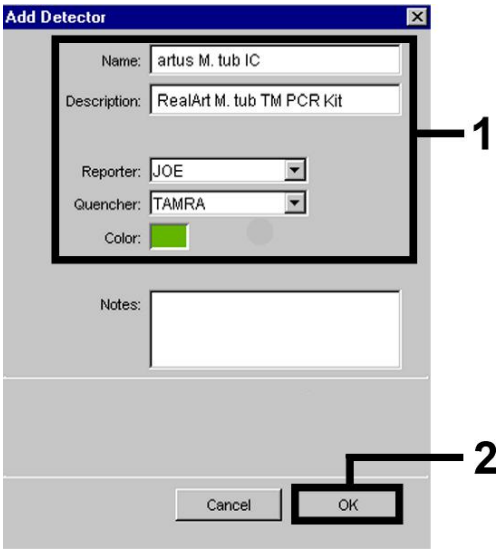

Fig. 18: Creating the IC specific detector (*Detector Manager*).

For the detection of *M. tuberculosis* complex DNA, define the reporter/ quencher combination **FAM/Non Fluorescent** in the now appearing window. For the detection of the *Internal Control*, select the combination **JOE/TAMRA** (as shown in Fig. 17 and Fig. 18). By confirming the input data (*OK*), return to the *Detector Manager*. Mark the newly created detectors and transfer each

selection to the *Setup* level by clicking on the option *Copy to Plate Document* (see Fig. 19). Close the window (*Done*).

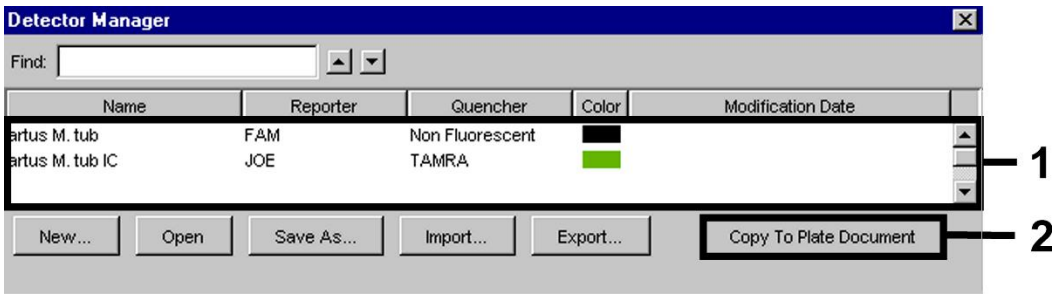

Fig. 19: Selecting the detectors (*Detector Manager*).

8.5.3.3 Assigning the Necessary Information to the Plate Positions

After having closed the *Detector Manager* (*Done*) the detectors selected under 8.5.3.2 are found on the *Setup* level (*Well Inspector*) listed in a table (see Fig. 20).

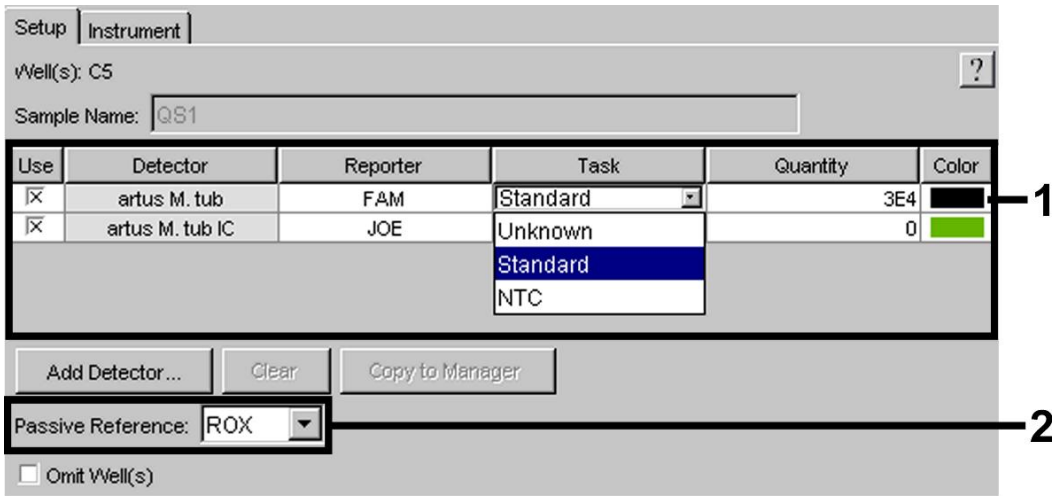

Fig. 20: Assigning the necessary information to the plate positions.

Mark the plate positions reserved for the detection of *M. tuberculosis* complex DNA. Assign the selected detectors to these positions by activating the *Use* option of both detectors by clicking on them, upon which a cross will appear. For the denomination of each single reaction, select the corresponding position on the plate and enter the name (*Sample Name*). Please note that preparations with an identical *Sample Name* and an identical detector

assignment will be identified as replicates by the software and will be averaged with respect to the quantified pathogen load. Then select the corresponding function (*Task*) for each sample type according to the following table:

Sample Type	Function (*Task*)	Concentration (*Quantity*)	Reporter	Quencher
Sample	Unknown	-	FAM	Non Fluorescent
Non-template control	NTC	-	FAM	Non Fluorescent
Standard	Standard	see **1. Contents**	FAM	Non Fluorescent

To generate a standard curve, use all supplied quantitation standards (*M. tuberculosis TM QS 1 - 4*) per PCR run and enter the corresponding concentrations (see **1. Contents**) for each standard (*Quantity*). Please note that for a PCR run with the *RealArt™ M. tuberculosis TM PCR Kit*, **ROX** has to be set as a passive reference (*Passive Reference*). The equal distribution of the ROX dye in all PCR preparations of a lot by mixing of the *M. tuberculosis TM Master* guarantees the recognition and calculation of tube-to-tube variations (fluorescence differences between several PCR preparations) by means of the *Sequence Detection Software* (normalization).

8.5.3.4 Creating the Temperature Profile

To create a temperature profile, switch from the *Setup* level to the *Instrument* level in the software. Enter the temperature profile specific for the detection of *M. tuberculosis* complex DNA according to Fig. 21. Make sure that the reaction volume for the detection of *M. tuberculosis* complex DNA by using the *RealArt™ M. tuberculosis TM PCR Kit* is set to 25 µl. The option *9600 Emulation* should be activated, the pre-settings of the *Ramp* time and the *Auto Increment* should remain unchanged (*Ramp Time*: 0:00, *Auto Increment*: 0.0°C, 0.0 Seconds).

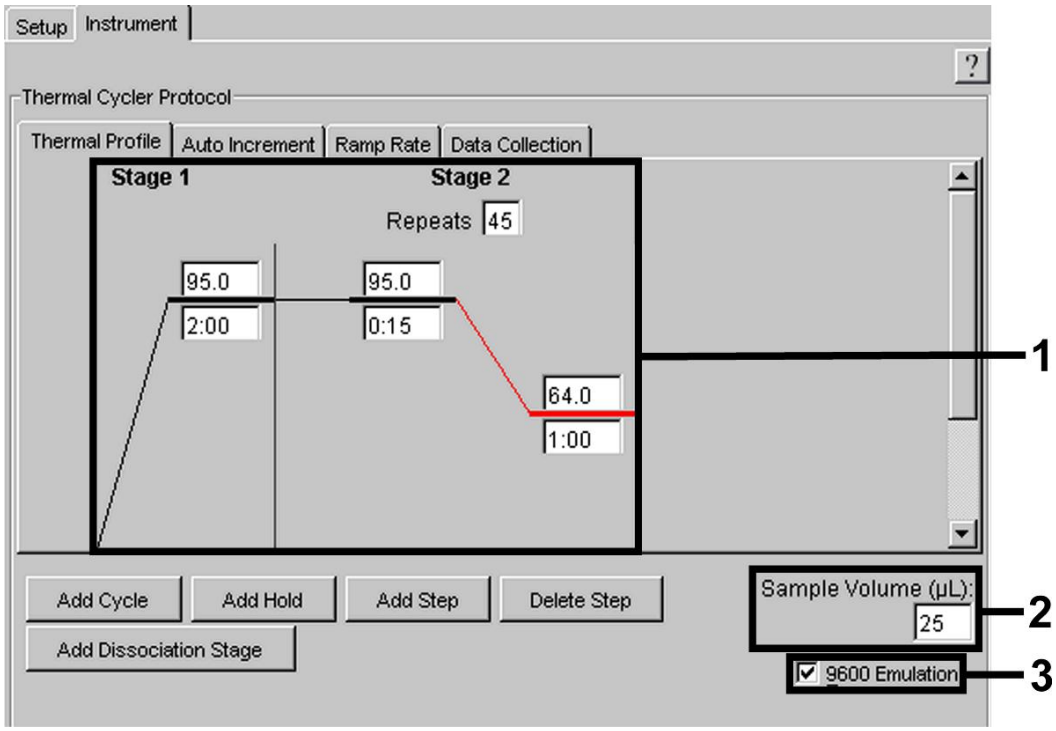

Fig. 21: Creating the temperature profile.

In addition, the *Instrument* level contains the option *Data Collection*. By selecting this option the window depicted in Fig. 22 is opened. Each ramp and each plateau temperature shows a *Data Collection Icon*, which illustrates the data being collected at this stage of the run. Remove all symbols except the one for the *Annealing-Extension* step (*Stage2*/Step2) by clicking on them in order to exclude unnecessary fluorescence measurements. The total run time and the data quantity will thus be kept at a minimum.

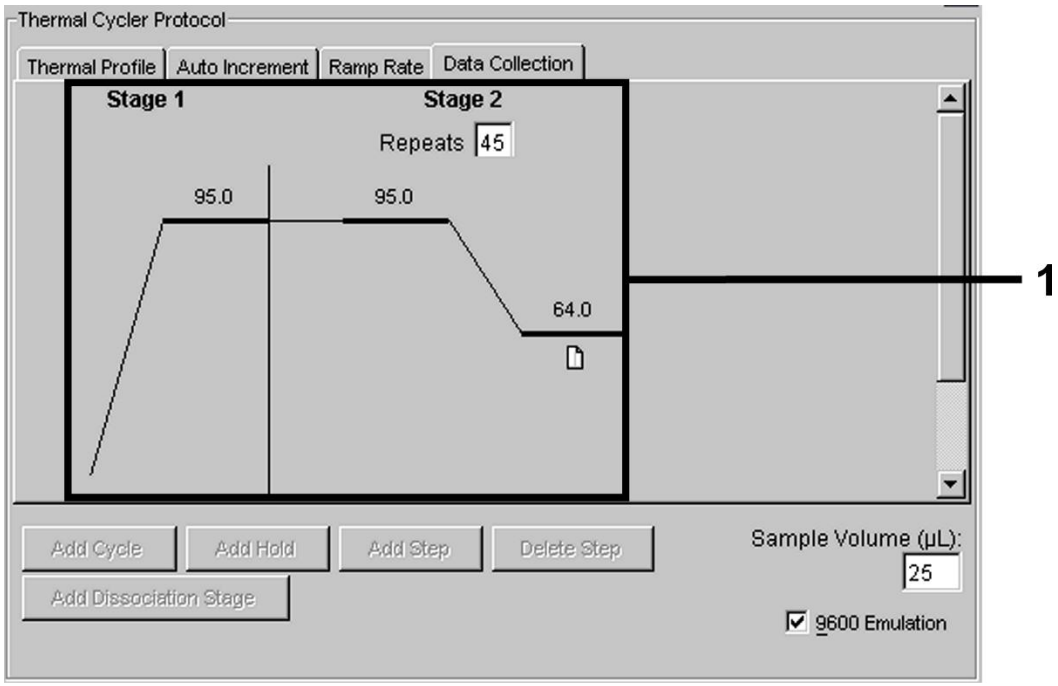

Fig. 22: Data collection.

8.5.3.5 Saving the PCR Run

Save the settings (*Setup*) as a template in order to make use of them again later in a modified or unchanged form. By saving the *Template* file as an *ABI PRISM®SDS Template Document* (**.sdt*) in the *Template Directory* ([*D:*]*Program Files*\ *Applied Biosystems**SDS 2.1**Templates*, created by Applied Biosystems) this file may be selected directly in the *New Document* window from the *Template* list. Copies stored in other folders have to be opened via *Browse*. Before starting the PCR run, save it again as an *ABI PRISM® SDS Document* (**.sds*) in order to guarantee the saving of the data that will be collected during the course of the PCR.

8.5.3.6 Starting the PCR Run

Start the PCR run by selecting the option *Start* from the menu item *Instrument* or the field *Start* on the *Analysis* level.

9. Data Analysis

A valid calibration of the dyes (***Pure Spectra Component File***) and the background (***Background Component File***) is necessary when putting the instruments into operation. These calibration files are required for the exact calculation of the results as follows:

All interfering signals generated by the instruments which influence the measurement are eliminated by the *Sequence Detection Software* of the *ABI PRISM® Sequence Detection Systems* by means of the *Background Component File*.

Furthermore, interferences appear during multicolour analyses between the emission spectra of the single fluorescence dyes. The software of the *ABI PRISM® SDS* compensates these interferences by calculations using the spectrum data of the individual dyes stored in the *Pure Spectra Component File*. The software uses the same file for the assignment of the fluorescence data comprising the entire measurable spectrum collected in the course of the PCR to the programmed detectors. The fluorescence data of the individual dyes are subsequently divided by the value obtained for the signal of the passive reference (ROX) in order to account for tube-to-tube variations (fluorescence differences between several PCR preparations). The thus normalized signals may then be evaluated by means of the *Amplification Plot*.

The calibration files used for the evaluation of a PCR run are automatically stored with the saving of the run. If no **calibration files** are installed, please create these files according to the instructions given in the *ABI PRISM® SDS User Guide/Manual.*

If more than one RealArt™ TM PCR system is integrated in your PCR run (note temperature profile!), these assays should be analysed separately. Samples with an identical *Sample Name* and an identical detector assignment will automatically be identified as replicates by the *ABI PRISM® 7000* and *7900HT SDS Software* and will be averaged with respect to the quantified pathogen load.

The following results are possible:

1. A FAM fluorescence signal is detected.

   The result of the analysis is positive: The sample contains DNA of one or more members of the *M. tuberculosis* complex.

   In this case, the detection of a JOE fluorescence signal (*Internal Control*) is dispensable, since high initial concentrations of *M. tuberculosis complex* DNA (positive FAM fluorescence signal) can lead to a reduced or absent fluorescence signal of the *Internal Control* (competition).

2. No FAM fluorescence signal is detected. At the same time, a JOE fluorescence signal from the *Internal Control* appears.

   In the sample no DNA of members of the *M. tuberculosis* complex is detectable. It can be considered negative.

   In the case of a negative *M. tuberculosis* complex PCR the detected signal of the IC rules out the possibility of PCR inhibition.

3. Neither a FAM fluorescence signal nor a JOE fluorescence signal is detected.

   No diagnosis can be concluded.

   Information regarding error sources and their solution can be found in **10. Troubleshooting**.

Examples of positive and negative PCR reactions are given in Fig. 23 / 24 (***ABI PRISM® 7000 SDS***), 25 / 26 (***ABI PRISM® 7700 SDS***) and 27 / 28 (***ABI PRISM® 7900HT SDS***).

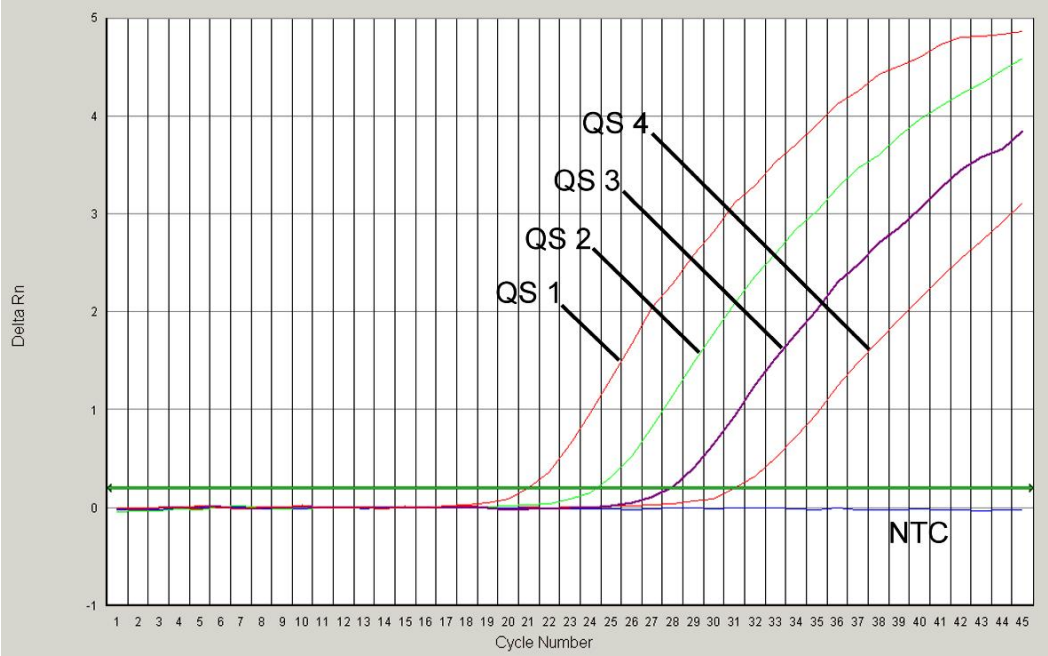

Fig. 23: Detection of the quantitation standards (*M. tuberculosis TM QS 1 - 4*) by measurement of a FAM fluorescence signal (***ABI PRISM® 7000 SDS***). NTC: non-template control.

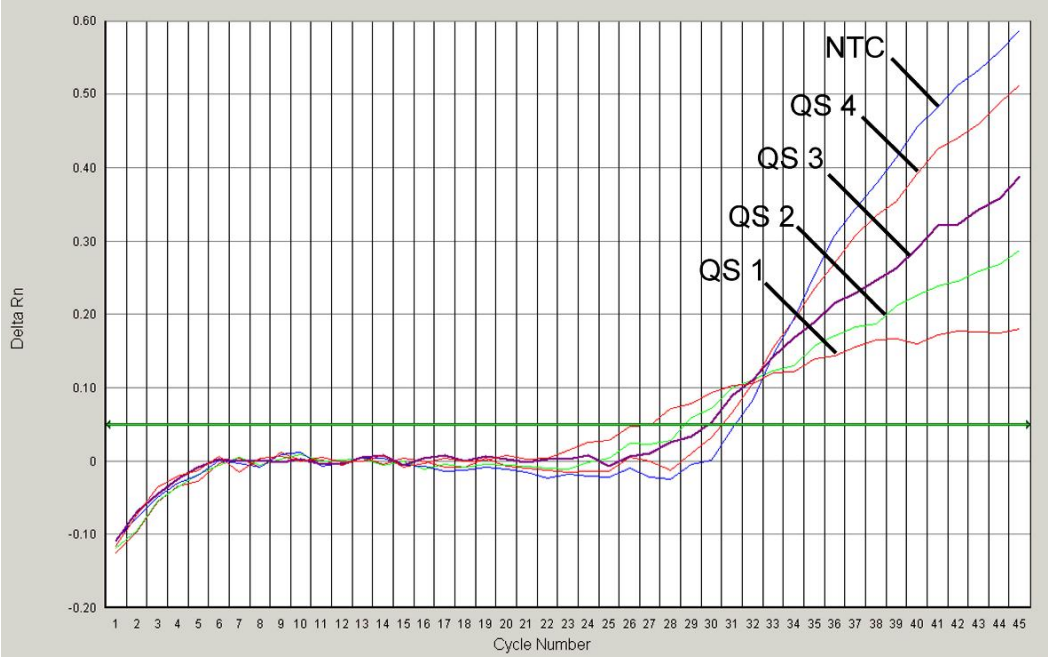

Fig. 24: Detection of the *Internal Control* (IC) by measurement of a JOE fluorescence signal (***ABI PRISM® 7000 SDS***) in parallel with a simultaneous amplification of the quantitation standards (*M. tuberculosis TM QS 1 - 4*). NTC: non-template control.

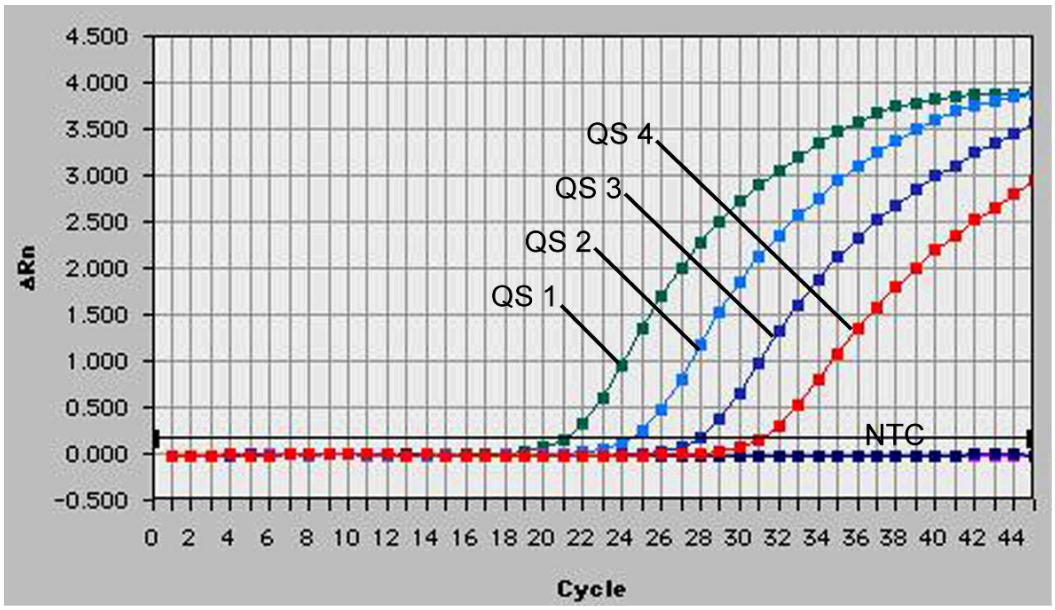

Fig. 25: Detection of the quantitation standards (*M. tuberculosis TM QS 1 - 4*) by measurement of a FAM fluorescence signal (***ABI PRISM® 7700 SDS***). NTC: non-template control.

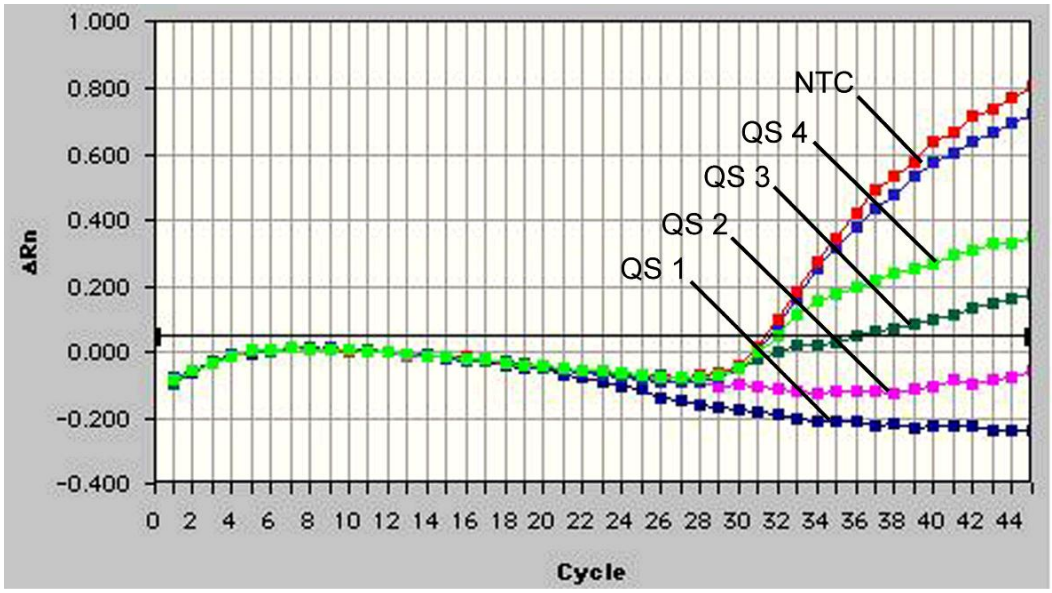

Fig. 26: Detection of the *Internal Control* (IC) by measurement of a JOE fluorescence signal (***ABI PRISM® 7700 SDS***) in parallel with a simultaneous amplification of the quantitation standards (*M. tuberculosis TM QS 1 - 4*). NTC: non-template control.

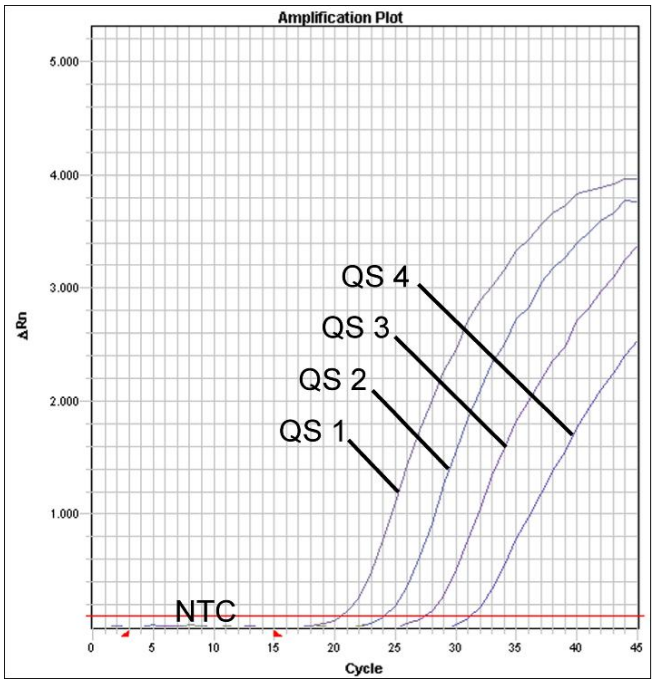

Fig. 27: Detection of the quantitation standards (*M. tuberculosis TM QS 1 - 4*) by measurement of a FAM fluorescence signal (***ABI PRISM® 7900HT SDS***). NTC: non-template control.

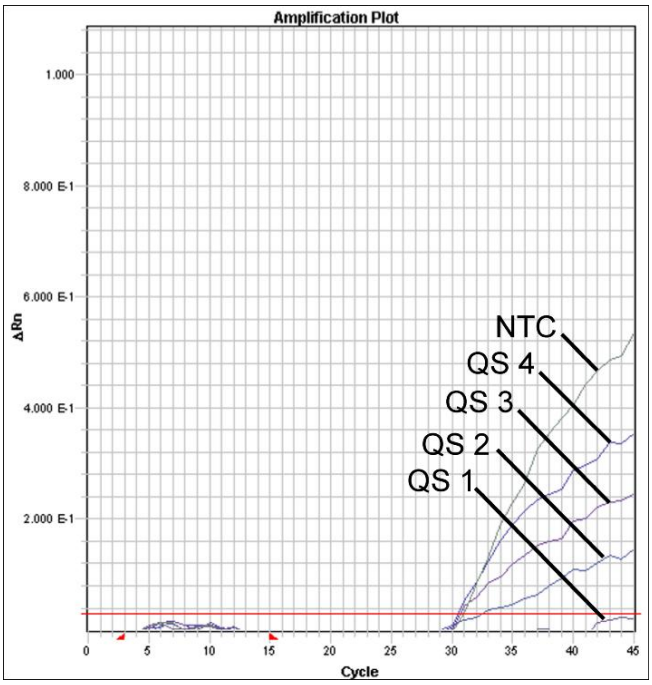

Fig. 28: Detection of the *Internal Control* (IC) by measurement of a JOE fluorescence signal (***ABI PRISM® 7900HT SDS***) in parallel with a simultaneous amplification of the quantitation standards (*M. tuberculosis TM QS 1 - 4*). NTC: non-template control.

10. Troubleshooting

No FAM fluorescence signal with positive controls (*M. tuberculosis TM QS 1 - 4*):

- Incorrect programming of the *ABI PRISM® Sequence Detection Systems*.
  - ➔ Repeat the PCR with corrected settings.

Weak or no signal of the *Internal Control* (JOE fluorescence signal) and simultaneous absence of a FAM fluorescence signal for the specific *M. tuberculosis* complex PCR:

- The PCR conditions do not comply with the protocol.
  - ➔ Repeat the PCR with corrected settings.
- The settings used for the data analysis under *Options* (*Extension Phase Data Extraction*) do not correspond to the settings of the *Data Collection* (see *ABI PRISM® 7700 SDS*, **8.5.2.4 Creating the Temperature Profile**).
  - ➔ Analyse the PCR run with corrected settings and repeat the data analysis (*Analysis*).
- The *M. tuberculosis TM Master* and/or the *M. tuberculosis TM IC* have been thawed and frozen too often.
  - ➔ Please mind the storage conditions given in **2. Storage**. Repeat the PCR using a new *M. tuberculosis TM Master*.
- The *M. tuberculosis TM Master* has been kept at +4°C for longer than five hours.
  - ➔ Please mind the storage conditions given in **2. Storage**. Repeat the PCR using a new *M. tuberculosis TM Master*.
- The PCR was inhibited.
  - ➔ Make sure that you use a recommended isolation method (see **8.1 DNA Isolation**) and stick closely to the manufacturer's instructions.
- DNA was lost during extraction.
  - ➔ Make sure that you use a recommended isolation method (see **8.1 DNA Isolation**) and stick closely to the manufacturer's instructions.

If you have any further questions or if you encounter problems, please contact our technical support at techsupport@artus-biotech.com.

11. Specifications

11.1 Sensitivity

In order to determine the sensitivity of the *RealArt™ M. tuberculosis TM PCR Kit*, a dilution series has been set up from 10 to nominal 0.05 *M. tuberculosis* copy equivalents♦/µl. It was subsequently analysed by means of the *ABI PRISM® 7000*, *7700* and *7900HT Sequence Detection System* with the help of the *RealArt™ M. tuberculosis TM PCR Kit*. Testing for all instruments was carried out on three different days on eight replicates. The results were determined by a probit analysis. A graphical illustration of the probit analysis (*ABI PRISM® 7000 SDS*) is shown in Fig. 29.

Detection limit (p = 0,05)	
ABI PRISM® 7000 SDS	1.0 copy/µl
ABI PRISM® 7700 SDS	1.0 copy/µl
ABI PRISM® 7900HT SDS	0.6 copies/µl

This means that there is a 95 % probability that 1 copy/µl (*ABI PRISM® 7000 SDS*), 1 copy/µl (*ABI PRISM® 7700 SDS*) and 0.6 copies/µl (*ABI PRISM® 7900HT SDS*) will be detected.

♦The standard is a cloned PCR product, the concentration of which has been determined by absorption and fluorescence spectroscopy.

Probit analysis: *M. tuberculosis* (*ABI PRISM® 7000 SDS*)

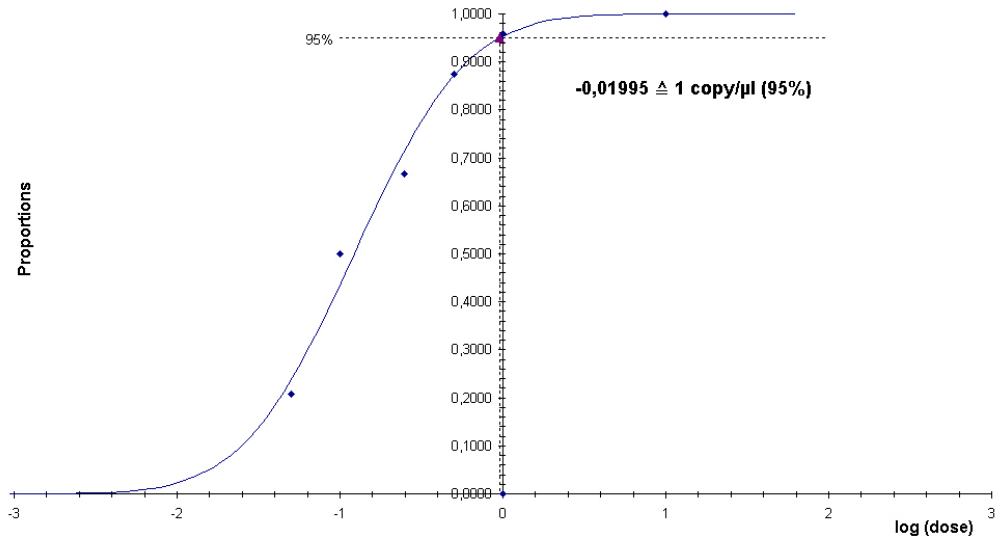

Fig. 29: Sensitivity of the *RealArt™ M. tuberculosis TM PCR Kit* (*ABI PRISM® 7000 SDS*).

11.2 Specificity

The specificity of the *RealArt™ M. tuberculosis TM PCR Kit* is first and foremost ensured by the selection of the primers and probes, as well as the selection of stringent reaction conditions. The primers and probes are checked for possible homologies to other known sequences by sequence comparison analysis. The detectability of all members of the *M. tuberculosis* has thus been ensured.

Moreover, the specificity was validated with 90 different *M. tuberculosis* complex negative samples (30 sputum, 30 BAL and 30 bronchial secretion samples). These did not generate any signals with the *M. tuberculosis* complex specific primers and probes, which are included in the *RealArt™ M. tuberculosis TM PCR Kit*.

To determine the specificity of the *RealArt™ M. tuberculosis TM PCR Kit* the control group listed in the following table (Table 1) has been tested for cross-reactivity. None of the tested pathogens has been reactive.

Table 1 (part I): Testing the specificity of potential cross-reactive pathogens.

Control Group	*M. tuberculosis* (FAM)	IC (JOE)
Actinomyces israelii	-	+
Aeromonas hydrophila	-	+
Bordetella pertussis	-	+
Candida albicans	-	+
Chlamydia trachomatis	-	+
Chlamydia pneumoniae	-	+
Citrobacter freundii	-	+
Corynebacterium diphtheriae	-	+
Corynebacterium jeikeium	-	+
Cryptococcus neoformans	-	+
Eikenella corrodens	-	+
Enterobacter aerogenes	-	+
Enterobacter cloacae	-	+
Enterococcus faecalis	-	+
Enterococcus faecium	-	+
Escherichia coli	-	+
Fusobacterium nucleatum ssp. polymorphum	-	+
Haemophilus influenzae	-	+
Haemophilus parainfluenzae	-	+
Klebsiella pneumoniae	-	+
Lactobacillus acidophilus	-	+
Mycobacterium avium ssp. avium	-	+
Mycobacterium celatum	-	+
Mycobacterium chelonae	-	+
Mycobacterium fortuitum	-	+
Mycobacterium gordonae	-	+
Mycobacterium intracellulare	-	+
Mycobacterium kansasii	-	+
Mycobacterium lentiflavum	-	+
Mycobacterium malmoense	-	+
Mycobacterium marinum	-	+
Mycobacterium scrofulaceum	-	+
Mycobacterium szulgai	-	+

Table 1 (part II): Testing the specificity of potential cross-reactive pathogens.

Control group	*M. tuberculosis* (FAM)	IC (JOE)
Mycobacterium ulcerans	-	+
Mycobacterium xenopi	-	+
Neisseria gonorrhoeae	-	+
Neisseria meningitidis	-	+
Nocardia asteroides	-	+
Nocardia brasiliensis	-	+
Nocardia farcinia	-	+
Nocardia otitidiscaviarum	-	+
Peptostreptococcus productus	-	+
Porphyromonas gingivalis	-	+
Prevotella denticola	-	+
Propionibacterium acnes	-	+
Pseudomonas aeruginosa	-	+
Salmonella enteritidis	-	+
Salmonella typhi	-	+
Staphylococcus aureus	-	+
Staphylococcus epidermidis	-	+
Streptococcus agalactiae	-	+
Streptococcus pyogenes	-	+
Streptococcus mutans	-	+
Streptococcus pneumoniae	-	+
Streptomyces venezuelae	-	+
Veillonella parvula	-	+
Xanthomonas maltophilia	-	+

11.3 Precision

The precision data of the *RealArt™ M. tuberculosis TM PCR Kit* allow the determination of the total variance of the assay. The total variance consists of the **intra-assay variability** (variability of multiple results of samples of the same concentration within one experiment), the **inter-assay variability** (variability of multiple results of the assay generated on different instruments of the same type and different operators) and the **inter-lot variability** (variability of multiple results of the assay using various lots). The data obtained were used to determine the standard deviation, the variance and the coefficient of variation for the specific and the IC system.

Precision data of the *RealArt™ M. tuberculosis TM PCR Kit* have been collected using the quantitation standard of the lowest concentration (QS 4; 30 copies/µl). Testing was performed with eight replicates. The precision data were calculated on basis of the Ct values of the amplification curves (Ct: threshold cycle, Table 2). In addition, precision data for quantitative results in copies/µl were determined using the corresponding Ct values (Table 3). Based on these results, the overall statistical spread of any given sample with the mentioned concentration is 1.41 % (Ct) or 10.23 % (conc.), for the detection of the *Internal Control* 2.59 % (Ct). These values are made up of the totality of all single values from the determined variabilities.

Table 2: Precision data on basis of the Ct values.

M. tuberculosis	Standard Deviation	Variance	Coefficient of Variation [%]
Intra-assay variability: *M. tuberculosis TM QS 4*	0.10	0.01	0.34
Intra-assay variability: *Internal Control*	0.29	0.09	0.99
Inter-assay variability: *M. tuberculosis TM QS 4*	0.12	0.01	0.37
Inter-assay variability: *Internal Control*	0.41	0.17	1.35
Inter-lot variability: *M. tuberculosis TM QS 4*	0.54	0.29	1.75
Inter-lot variability: *Internal Control*	0.80	0.64	2.57
Total variance: *M. tuberculosis TM QS 4*	0.44	0.19	1.41
Total variance: *Internal Control*	0.79	0.63	2.59

Table 3: Precision data for quantitative results in copies/µl.

M. tuberculosis	Standard deviation	Variance	Coefficient of variation [%]
Intra-assay variability: *M. tuberculosis TM QS 4*	2.17	4.72	7.22
Inter-assay variability: *M. tuberculosis TM QS 4*	2.37	5.63	7.89
Inter-lot variability: *M. tuberculosis TM QS 4*	3.96	15.65	13.08
Total variance: *M. tuberculosis TM QS 4*	3.08	9.51	10.23

11.4 Robustness

The verification of the robustness allows the determination of the total failure rate of the *RealArt™ M. tuberculosis TM PCR Kit.* 30 *M. tuberculosis* complex negative samples of sputum, BAL and bronchial secretion were spiked with 3 copies/µl elution volume of *M. tuberculosis* control DNA (threefold concentration of the analytical sensitivity limit). After extraction using the QIAamp DNA Mini Kit (QIAGEN; see **8.1 DNA Isolation**) these samples were analysed with the *RealArt™ M. tuberculosis TM PCR Kit.* For all *M. tuberculosis* samples the failure rate was 0 %. In addition, the robustness of the *Internal Control* was assessed by purification and analysis of 30 *M. tuberculosis* complex negative sputum, BAL and bronchial secretion samples. The total failure rate was 0 %. Inhibitions were not observed. Thus, the robustness of the *RealArt™ M. tuberculosis TM PCR Kit* is ≥ 99 %.

11.5 Reproducibility

Reproducibility data permit a regular performance assessment of the *RealArt™ M. tuberculosis TM PCR Kit* as well as an efficiency comparison with other products. These data are obtained by the participation in established proficiency programs.

11.6 Diagnostic Evaluation

Currently, the *RealArt™ M. tuberculosis TM PCR Kit* is undergoing a series of evaluation studies.

12. Product Use Limitations

- All reagents may exclusively be used in *in vitro* diagnostics.
- The product is to be used by personnel specially instructed and trained in the *in vitro* diagnostics procedures (EN375) only.
- Compliance with the user manual is required.
- Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.

13. Explanation of Symbols

Use by

Batch code

Manufacturer

Catalogue number

Temperature limitation

QS

Quantitation Standard

IC

Internal Control

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